REMARKS

The claims pending and under examination in this application are claims 23-25 and 27-41. Claim 26 has been cancelled and new claim 42 is added by this Amendment.

The rejection of claims 25 and 27-41 under 35 USC § 112, second paragraph, is respectfully traversed.

The meaning of the term "anti-sublimating agent" has been clarified to make explicit the fact that this agent prevents sublimation of the dinitroaniline. The inventors of the present invention have found that sublimation is a specific process wherein the trifluralin compound passes directly to the gaseous phase if an anti-sublimating agent is not present in the solution described in the process set out in amended claim 25. The anti-sublimation agent has been found to be a sugar, such as trehalose or mannitol. When such an agent is used, it is possible to avoid separation by sublimation of the trifluralin composition during the preparation of liposomal formulations for use in treating humans or animals.

Claim 27 has been amended so as to depend from claim 25, which has not been cancelled.

In claim 34, the terms "small" and "rest" have been amended to make their meanings clearer. The meaning of these terms has been set forth to define the preferred quantities or volumes of aqueous solution which are set forth in the hydration in Step 1 of claim 25. A new claim, 42 further defines the volume of aqueous solution in a range of first 5 to 30 % of the final volume of aqueous solution in Step 1 and then following this step by addition of the remainder of 70 - 95 % of final volume after a 30-minute period of rest. The designation of cholesterol, by spelling out the word completely, has been changed to adopt the Examiner's suggestion in the last line of page 2 of the

Office Action. Likewise, the Examiner's suggestion at the top of page 3 has been adopted, whereby, in claim 38, the word "comprises" has been changed to "is."

The term "dinitroaniline" pesticide is now expressed in the amended claim 39, so that it refers back to the same terminology as that found in claim 25, at lines 2 and 3.

The rejection of claims 40 and 41 under 35 USC § 101, for lack of defined steps in the process, is respectfully traversed in view of the amendments to claims 40 and 41 offered in this Response. The process steps in the amended claims 40 and 41 are now set forth clearly. Thus, the words "use" or "using" have been deleted from claims 40 and 41.

The rejection of claims 23, 24 and 39 under 35 USC § 102(b), as anticipated by WO 95/31970 of record, is respectfully traversed. This reference is directed to a method of preparing a pre-determined active agent stock solution for liposomal micro encapsulation of active agents for agricultural uses. This reference is not directed to pharmaceutical uses of liposomal compositions. In fact, on page 1 of the specification of this reference, at lines 20-23, it states that pesticides, such as herbicides, fungicides, insecticides, bactericides and other active agents and compounds are applied periodically in the home, agriculture and other places and can be dangerous to humans. This reference also teaches a new method of producing liposomal micro encapsulated agricultural active agents. On page 2 at lines 4-7, this reference states that "nothing in the prior art either suggests, teaches or discusses the use of liposomal micro encapsulation techniques to active agents such as pesticides in agricultural formulations." There is nothing in this reference to suggest that two (2) distinct populations of liposomal formulations can, or should, be used with mean diameters bigger and lower than 100 nm as is claimed and described in the present application.

The present invention as claimed in claims 23, 24 and 39 relates to liposomal formulations of different sizes and different physical and chemical properties, different lipid and drug ratios, and different drug quantities. The liposomal formulations known from the prior art literature have continuous size distributions and there is no teaching in the prior art of how to quantify previously to their administration, the amount of active drug in each of the populations, bigger than 400 nm and lower than 100 nm or in any other size range.

These two different pre-prepared and pre-quantified liposomal formulations of different sizes and different physical and chemical properties having different targets and the final mixture can be programmed and prepared in such a way that the two liposomal populations can simultaneously reach the therapeutic effective doses for each of those targets.

When one prepares a liposomal formulation that is not sized, a Gaussian curve of size distribution results. This distribution includes 100 nm and 400 nm and many others from less than 50 nm up to several microns.

An example of the following method has been developed to prepare a liposomal formulation able to reach simultaneously the following targets:

1. Liver and spleen;
2. Bone marrow and skin;
3. Target 1 can be reached by 400 nm liposomes; and
4. Target 2 can be reached by 100 nm liposomes.

To treat a disease, it is necessary to have the therapeutic dose at the target site. How can we reach the therapeutic dose in the form of 400 nm liposomes to target 1, and the therapeutic dose in

the form of 400 nm liposomes to target 1, and the therapeutic dose in the form of 100 nm liposomes at target 2 at the same time?

The claims have been amended to clearly define the subject matter thereof, and recite the process steps and use proper format with antecedent bases, etc. Accordingly, the rejection of the claims under 35 U.S.C. § 112 is rendered moot by these amendments. Reconsideration and allowance of the claims is respectfully solicited.

At paragraph 2, page 2 of the Office Action, responsive to the Examiner's question, if no anti-sublimating agent is added prior to lypohilization (the dehydration step) trifluralin will be removed from the formulations during this step. So it is imperative to add an anti-sublimating agent. This anti-sublimating agent can be a sugar but some of them are better for this purpose than others (page 6, line 26 of the present application). As can be seen on page 8, line 25 of the present application, trehalose is used in all the hydrating solutions in order to be present and protect trifluralin for being removed during the dehydration step. The rejection under 35 USC § 102(b) over WO 95/31970 is respectfully traversed.

The described method refers to processes of production of a saturated organic solution of lipid and an active agent that, upon addition of water, would become a liposomal formulation. This method does not produce liposomals. Liposomals can only be formed if the organic solvent is removed by any means. The only possible systems that can come out of that method are micelles.

By definition, liposomals are lipid vesicles consisting of one or more concentric sealed bi-layers, dispersed in an aqueous environment. The presence of organic solvents will make it impossible to cause the formation of a bi-layer structure.

Concerning novelty in the present application, any person skilled in the art knows how to prepare liposomals. These liposomals will obey a broad Gaussian distribution around the selected size for the preparation. The novelty of this application is that it predefines two different sizes according to the desired target organs (page 8, lines 1 to 9 of the present application). Liposomal formulations achieved by mixing two distinct liposomal populations with two distinct average sizes, obeying two distinct Gaussian distributions are never referred to or presented in the prior art.

Reconsideration of the rejection of the claims to the liposomal formulation and the method of preparing the formulation and using it is respectfully solicited in view of the foregoing arguments and amendments.

The inventive step is that in spite of the thousands of publications in the field that are presented every year, for the first time the inventors of the present invention have discovered that mixing two distinct populations with predefined average sizes to reach different selected organs would be very beneficial to the treatment of diseases that could not be treated with only one liposomal population. It is the case of leishmaniosis. The disease can be present in many organs such as liver, spleen, bone marrow and skin. Liver and spleen can be targeted with a liposomal population of mean size of 400nm that will not reach the bone marrow and the skin. In order to reach the bone marrow and the skin, simultaneously with the liver and the spleen, a liposomal population with the mean size of 50nm should be mixed with the one that has a 400nm mean size, ensuring in this way that the liver, spleen, bone marrow and skin will be able to reach the necessary amount of liposomals to achieve a therapeutic effect.

In order to better illustrate the problem discussed above, the following example is offered:

Assuming that the therapeutic dose to reach target 1 is 100 mg of liposomal drug, and to reach target 2 the therapeutic dose is also 100 mg of liposomal drug.

Case A -Non-sized liposomes -How much of the total liposomes can we administer? 200 mg is not enough since that amount will be distributed by all sizes and not only by 100 nm and 400 nm sizes. How do we plan the experiments? We do not know. If we add too little, we will have a sub-therapeutic dose (non effective); if we add too much we can have. toxicity.

Case B -With the two separated formulations, the planning of the experiments is easy: A mix formulation is prepared containing 100 mg of drug in liposomes of size 100 nm plus 100 mg of drug in liposomes of size 400 nm. Each are mixed in together to reach the right concentration of each respective target.

Please note that (D/L)f means the drug to lipid ratio on the prepared formulations and that I. E. means incorporation efficiency. As can be seen in the above figures only when the formulations were prepared with trehalose, trifluralin was kept in the formulation during the freeze-drying process.

Applicants are not claiming lyophilization, nor are they claiming cryoprotectant agents, nor are they claiming long time storage, and they are not claiming the dehydration-rehydration process. The antisublimating agent has a different task than the cryoprotectant agent.

The cryoprotectant agents, which are well-known, will avoid the formation of ice crystals during the lyophilization process. The antisublimating agent will avoid drug sublimation both from suspension of liposomes and from powder. Not all cryoprotectant agents are anti-sublimating agents for these drugs. Applicants have invented a new, unobvious process since

they are not aware of the use of an anti-sublimating agent for this drug, besides those found by Applicants.

Thus, claims 25, 27-38 and 42 are deemed patentable over the art of record.

If requested, Applicants are prepared to submit a Declaration under 37 CFR §1.132 to support the facts stated in the above argument.

In view of the foregoing argument, reconsideration of the rejection of claims 23, 24 and 39 as being anticipated by WO 95/31970 of record is respectfully solicited.

The rejection of claims 23-24 and 39-41 under 35 USC § 103(a) as being unpatentable over Applicants statements of prior art in view of Steck (4,186,183) and Rao (4,594,241), individually, in any combination thereof, is respectfully traversed. Applicants concede that the herbicide, trifluralin is a well-know anti-leishmania drug. It is assumed that this is the statement of the prior art as referred to by the Examiner in this rejection under 35 USC § 103(a). It is further assumed that the reference made to page 6 of Applicants' last Response, dated June 26, 2002, is not in question as to Applicants' admitted statements of the prior art. Page 6 of Applicants' June 26, 2002 Response does not address a Gaussian type distribution, which is described at the bottom of page 4 and again at the top of page 5 of the Office Action. On page 7 of the June 26, 2002 Response, in the third full paragraph, there is a discussion of Gaussian type distribution. The full text is as follows:

> Concerning novelty in the present application, any person skilled in the art knows how to prepare liposomes. These liposomes will obey a broad Gaussian distribution around the selected size for the preparation. The novelty of this application is that it pre-defines two different sizes according to the desired target organs (page 8, lines 1-9 of the present application). Liposomal formulations achieved by mixing two distinct liposomal populations with two distinct average sizes, obeying two distinct Gaussian distributions are never referred to or presented in the prior art.

The Steck reference is directed to an anti-leishmanial agent incapsulated within liposomes and the liposome encapsulated drug is injected into the body. Steck, however, does not describe or indicate that trifluralin or related dinitroanline pesticides are in question. Instead, an anti-monial drug is suggested for treating leishmania.

Rao is also directed to antimony-containing anti-leishmaniasis drugs, which are encapsulated in a lipid-based liposomal formulation.

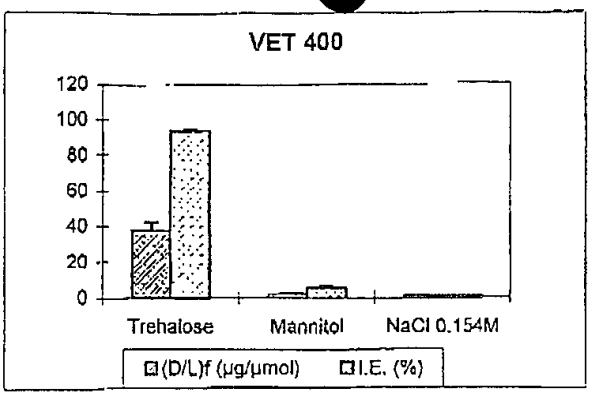
The rejection of claims 23-24 and 39-41 under 35 USC § 103(a) over Applicants' statements of prior art in view of Steck individually or in any combination with Rao with the prior art admitted by Applicants, is respectfully traversed for the foregoing reasons and because the formulation of two distinct populations of liposomal compositions of differing size, both above and below 100 nm for the specific purpose of treating ailments of humans or animals is not disclosed or even suggested in Rao, Steck, individually or in combination as secondary references supporting Applicants' own statements of the prior art and vice versa. As stated hereinabove, the present invention relates to a liposomal formulation made from two different pre-prepared and quantified liposomal formulations of different size and different physical and chemical properties, different lipid and drug ratios and different drug quantities. The liposomal formulations known from Steck and Rao have continuous size distributions and there is no teaching in the prior art of how to quantify previously to their administration, the amount of active drug in each of the populations bigger than 400 nm and lower than 100 nm, or in any other size range.

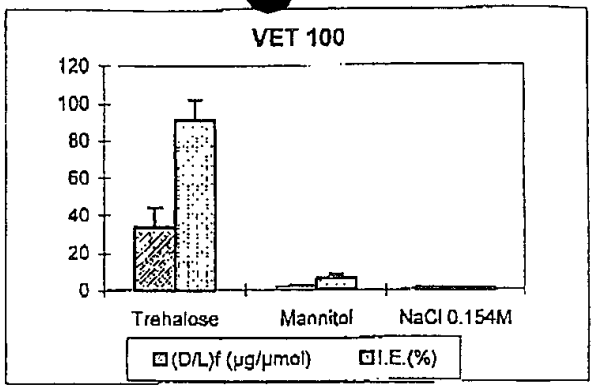
The rejection of claims 23-25 and 27-41 under 35 USC § 103(a) as being unpatentable over Applicants' statements of prior art in view of Steck, Rao, individually or in combination, as set forth in the Office Action on pages 5 and 6, and further in view of British Patent 2,002,319 or WO 95/31970,

cited above, is respectfully traversed for the foregoing reasons, as applied to claims 23, 24 and 39 against WO 95/31970 of record, and as applied against claims 23-24 and 39-41 under 35 USC § 103(a), as applied over Applicants' statements of prior art in view of Steck, Rao, individually or in combination. The Examiner states that neither Steck nor Rao, nor WO 95/31970 teach the dehydration of the liposomes and rehydration thereof. British Patent 2,002,319 teaches that liposomes can be dehydrated for storage as a stable powder and according thereto, such dehydrated powders may be stored for long periods and from which a dispersion of liposomes can be reconstituted. Dehydrating the liposomes of Steck or Rao or WO 95/31970 would have been obvious to one of ordinary skill in the art, because the British patent teaches that the liposomal powders can be stored for a long period. The rejection based upon this reasoning and upon these references is respectfully traversed for the reasons outlined above and in view of the following.

The use of an anti-sublimating agent in the process described and claimed in claim 25 and those claims dependent thereon is nowhere taught or even suggested in any of the prior art.

Volatility of dinitroaniline compounds such as trifluralin, oryzalin and ethalfluralin, for example, is well documented. This is a major problem, during manipulation of the formulation (where one can find trifluralin adsorbed to the parafilm that is used to cover flasks and erlenmeyers)or during lyophilization where all the trifluralin comes out of the samples. To prevent this from happening, certain exact types of agents are added. As an example to illustrate these findings, some of the applicants' results are presented where the same liposomal composition was prepared with antisublimating agents, respectively, without antisublimating agents (e.g., saline).





In view of the foregoing arguments and amendments offered in this Response, favorable action and allowance of the present application and of all claims herein, is respectfully solicited.

Should the Examiner wish to contact Applicants' representative, he may do so by telephoning Edward H. Valance, Reg. No. 19,896, at (703) 205-8000 in the Washington Metropolitan area.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two-month extension of time for filing a reply in connection with the present application, and the required fee of $410.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version With Marking To Show Changes

VERSION WITH MARKING TO SHOW CHANGES MADE

IN THE CLAIMS:

23. (Twice Amended) A liposomal formulation containing a [dinitro analine] dinitroaniline pesticidal agent incorporated in a pharmaceutical formulation comprising a mixture of at least two separate and distinct liposome formulations whose populations of particles [with distinct] have mean diameters respectively bigger and lower than 100 nm.

24. (Amended) The liposomal formulation according to claim 23, which comprises a mixture of pre-defined predefined populations of particles with mean diameters respectively bigger than 400nm and lower than 100nm.

25. (Thrice Amended) A process for the preparation of a final formulation composed [plurality] of distinct populations [of liposomal formulations of particles] with distinct mean diameters, containing [one] a dinitroaniline pesticide, which comprises the steps:

[(1)] 1. [obtention] obtaining of the liposomal formulations containing vesicles of dinitroaniline pesticide by hydration, with a solution containing an antisublimating agent of a lipidic film containing the dinitroaniline pesticide;

2. Obtaining different populations with well-defined diameters by a sizing step;

3. mixing the distinct populations;

[(2)] 4. lyophilization [and] dehydration of the dinitroaniline so obtained liposomal formulations; and

[(3)] 5.rehydration of the dehydrated liposomal formulations.

27. (Amended) The process according to claim [26] 25, which comprises performing the sizing step by extrusion of the vesicles under Pressure through porous membranes.

28. (Amended) The process according to claim 25, wherein the hydration is carried out by the addition of [a small amount] an aliquot portion of an aqueous solution, followed by the addition of the remaining volume of the aqueous solution, after a resting period.

29. (Amended) The process according to claim 28, which comprises using, in the hydration steps, a [non-saline] salt-free solution.

30. (Amended) The process according to claim 29, which comprises performing the rehydration steps with saccharose, trehalose, glucose or [any other sugar solution] mixtures thereof.

32. (Amended) The process according to claim [31] 25, which comprises mixing particles after sizing to yield a population of particles with diameters of, respectively, bigger and lower than 100 nm.

34. (Amended) The process according to claim [31] 25, which comprises performing the hydration by addition of a small amount of aqueous solution, namely 20% of the final volume, followed by addition of the rest of the volume, namely 80% of the final volume, after a 30-minute rest period.

35. (Amended) The process according to claim [31] 25, which comprises using in the hydration step a [non-saline] salt-free solution.

36. (Amended) The process according to claim [31] 25, which comprises performing rehydration [using] with a member selected from the group consisting of solutions of saccharose, trehalose, glucose [or another sugar] and mixtures thereof.

37. (Amended) The process according to claim [31] 25, which comprises using at least one of the lipids selected from the group consisting of distearoylphosphatidylcholine (DSPC), phosphatidylcholine (PC), cholesterol [(Chol) or] and cholesterol derivatives, sphingomielin (SM), dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylglycerol (DOPG), phosphatidylglycerol (PG), dimiristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), gangliosides, ceramides, phosphatidylinositol (PI), phosphatydic acid (PA), dicetylphosphate (DcP), dimiristoylphosphatidylglycerol (DMPG), stearylamine (SA), dipalmitoylphosphatidylglycerol (DPPG) and mixtures thereof.

38. (Amended) The process according to claim [31] 25, wherein the dinitroaniline pesticide is [comprises] trifluralin.

39. (Amended) A liposomal formulation [according to claim 23, when prepared] containing a dinitroaniline pesticide when prepared by [the] a process according to claim 25.

40. (Amended) A method of [using] applying the liposomal formulation as defined according to claim 23 [for] which comprises the treatment of disease in humans or animals, [which comprises] wherein administration of a therapeutic quantity of the [dinitroaniline] liposomal formulation is applied to humans or animals.

41. (Amended) [Use of liposomal formulations] Method for the preparation of a pharmaceutical [formulation] composition for the treatment [in] of humans or animals, [characterized by the use of] wherein a composition is provided containing a therapeutic [efficient] quantity of the pharmaceutical [formulation] composition containing [a] the dinitroaniline liposomal formulation prepared according to [claim 39] the process of claim 25.

Please add the following new claim 42:

42. The process according to claim 25, which comprises performing the hydration in step 1 by addition of 5-30% of final volume of aqueous solution, followed by addition of the remainder 70-95% of final volume, after a 30-minute period of rest.